

Quantitative evaluation of macrophage aggregates in brook trout *Salvelinus fontinalis* and rainbow trout *Oncorhynchus mykiss*

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ABSTRACT: Macrophage aggregates (MAs) occur in various organs of fishes, especially the kidney, liver and spleen, and contain melanin, ceroid/lipofuscin and hemosiderin pigments. They have been used as indicators of a number of natural and anthropogenic stressors. Macrophage aggregates occur in salmonids but are poorly organized, irregularly shaped, and are generally smaller than those in derived teleosts. These features complicate quantification, and thus these fishes have seldom been used in studies correlating MAs with environmental stressors. To alleviate these complications, we developed color filtering algorithms for use with the software package ImagePro Plus® (Media Cybernetics) that select and quantify pigmented area (i.e. colors ranging from gold to brown to black) in tissue sections. Image analysis results compared well with subjective scoring when tested on brook trout *Salvelinus fontinalis* and rainbow trout *Oncorhynchus mykiss* captured from high-elevation lakes or hatcheries. Macrophage aggregate pigments correlated positively with age and negatively with condition factor. Within individual fish, pigmentation correlated positively among organs, suggesting that the kidney, liver or spleen are suitable indicator organs. In age-matched fishes, MA pigments were not different between hatcheries and lakes in the organs examined. Between lakes, differences in pigments were observed in the kidney and spleen, but were not explained by age, condition factor, sex or maturation state. Our results indicate that quantification of the area occupied by MA pigments is an efficient and accurate means of evaluating MAs in salmonid organs and that organ pigmentation correlates with age and condition factor, as seen in studies with more derived fishes.

KEY WORDS: Macrophage aggregates · Brook trout · Rainbow trout · Fishes · Pigment · Ecotoxicology · Stress